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METHODS OF ANALYSIS FOR SOLANINE IN TUBERS OF SOLANUM TUBEROSUM

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April 1952

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THE UNIVERSITY OF ALBERTA

METHODS OF ANALYSIS FOR SOLANINE IN TUBERS OF SOLANUM TUBEROSUM

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

Faculty of Agriculture

Department of Plant Science

by

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EDMONTON, ALBERTA

April, 1952

Methods of Analysis for Solanine in Tubers of *Solanum tuberosum*

(Abstract)

The problem of bitterness in table potatoes caused by the glycoalkaloid solanine is causing some concern in the Province of Alberta. Before a comprehensive study of causal factors could be undertaken, it was essential to develop a relatively short method of quantitatively estimating the solanine content of a sample of tubers. The investigational work reported in this paper was carried out in an effort to obtain such a short analytical method.

No pure solanine could be obtained through commercial channels, so it was necessary for the author to isolate and purify the compound as a prerequisite to the investigation.

An analytical procedure was evolved, by an amalgamation of two existing methods, which was used as a Standard Method in this study. An electrophotometer was available which made possible the use of the proven colorimetric method of solanine estimation from the potato extract.

Unsuccessful attempts were made to estimate the solanine content of potatoes by: using qualitative re-

agents on fresh tuber slices; a simplification of the regular extraction procedure; measuring the fluorescent intensity of solanidine, a product of solanine acid-hydrolysis; and extraction from dried tuber material.

Late in the present investigation, a shortened analytical technique for solanine determination in potato tubers was developed. Use of this method enables the investigator to complete a duplicate analysis within 36 - 40 hours, which is about one-third as much time as that required by the older methods. Fresh tuber material was extracted in a Soxhlet extraction apparatus, using acidified 95 per cent ethyl alcohol as the solvent. The solanine content of this extract was estimated colorimetrically. Careful application of this procedure yields good results that are more consistent than those obtained using the Long Standard Method.

Statistical analysis of duplicate solanine determinations gave a "t" value of only 0.03 for thirty degrees of freedom. The standard error of the method = ± 0.154 mg. of solanine per 100 gm. of fresh tuber tissue. These figures strongly indicate the reliability of the method.

It is hoped that this shortened and simplified technique for solanine analysis of potato tubers will prove useful to other workers in this field.

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INTRODUCTION AND LITERATURE REVIEW

For various reasons, the variety Netted Gem is the most important potato variety grown commercially in the Province of Alberta. One of these reasons is that Alberta-grown "seed" of Registered and Certified Netted Gems commands a premium in Idaho, where this variety is very popular. Another reason for the popularity of this variety in Alberta is the fact that it is resistant to Common Scab and will normally produce a crop of clean, very attractive tubers.

This potato variety is not without its shortcomings. It is not the highest quality table potato that can be grown at this latitude, although its quality is rated as good. The reason that the author became particularly interested in the Netted Gem variety was the fact that consumers often complained about the bitter flavor of Alberta potatoes purchased through regular channels. These complaints have resulted in sales losses and could easily result in serious acreage reductions, with attendant hardships to the potato grower.

Experimental work to date has resulted in many statements that are often contradictory regarding causal factors. It has been quite generally agreed that the specific bitterness in question is caused by the glycoalkaloid

solanine. This complex is apparently present in all potato tubers, but normally comprises a very small proportion of the tuber. In some seasons, at least a portion of the crop will have a very acrid flavor, and upon analysis show a higher-than-average solanine content.

From a critical study of solanine research already reported, it is apparent that this problem is rather complex. The first study that suggested itself was an exhaustive attempt to abbreviate the long, tedious analytical methods in present use. It was almost essential to have at least a relatively short method of quantitative analysis before a really comprehensive study of causal factors could be commenced. The two most important analytical methods in use to date require from three to five days in order to complete duplicate analyses. Also, so many individual steps are involved that the probability of errors is very great.

Chemical Nature of Solanine

(a) Brief Description of Alkaloids -

Gortner (15) says that, because of their physiological properties, alkaloids have, for ages past, interested mankind. He further states that it seems probable either that the nitrogen bases (which include the alkaloids) represent metabolic products formed by the breaking down of amino acids in the plant or animal organism, or that amino acids enter into the synthesis of these compounds. The nitrogen bases, therefore, may be looked upon as either amino acid decomposition products, or products which have been derived from amino acids through some vital process.

It is only within the last few decades that the organic chemistry of certain of the alkaloids has been elucidated. Owing to the complex structure of the alkaloidal molecule (15), a study of the chemistry of the alkaloids is one of the most difficult fields of organic chemistry, and even today the structural formulae of several important drugs are uncertain.

It is difficult to formulate an exact definition of an alkaloid. The definition may be made so broad as to include all nitrogen-containing compounds, or so narrow as to leave out compounds with definite alkaloidal properties. Perhaps the following definition of Thompson, as quoted by

Gortner (15), is as satisfactory as any yet devised: "An alkaloid is a relatively complex organic chemical entity containing nitrogen, whose free base is alkaline in reaction and capable of neutralizing acids to form salts, whose free base is much less soluble in water than its salts, whose free base is much more soluble in ether, chloroform, benzol, etc., than its salts, and as a substance whose salts in solution release the free base upon the addition of alkalies."

According to Gortner (15), the question of origin and purpose of such compounds as the alkaloids, in the economy of a plant, is a question which will probably always remain a subject of speculation. We have no evidence as to the real significance of the alkaloids in the organs or tissues of a plant. Inasmuch as they do not occur in all forms of plant life, it is obvious that they are not essential to the life process of a plant. The great variability in amount from season to season and from locality to locality also indicates that they may possibly be regarded as by-products of the synthetic and metabolic activities of the plant (15, 19). This is the view of Pictet, as reviewed by Gortner (15), who believes that the nitrogen residues from protein utilized by the plant in its metabolic processes are resynthesized into alkaloids (by certain plants which possess such synthetic power), because in this form the alkaloids are less harmful to the plant than the direct decomposition products of the

proteins. It has also been suggested that alkaloids are a form of protection to the plant from marauding animals. In the opinion of the writer, it appears possible that the alkaloid content of certain plants may also form a means of protection from insect attack, with consequent survival of plant populations relatively high in a certain alkaloid.

(b) Is Solanine an Alkaloid?

Gortner (15) terms solanine a compound which may be considered as intermediate between the true alkaloids and other compounds containing a basic nitrogen group. He calls it an alkaloidal glycoside. Wolf and Duggar (29) refer to solanine as a glycoalkaloid, and Henry (16) treats solanine in the chapter on Steroid Alkaloids. This material certainly has a physiological action which is a property characteristic of the alkaloids.

(c) Suggested Structural Nature and Properties of Solanine -

Solanine occurs in the potato (Solanum tuberosum), and on acid hydrolysis yields one molecule each of glucose, galactose, and rhamnose, and a base, solanidine (15, 16, 24, 29). Various suggestions have been made as to the empirical formula of the compound. In 1937, Conner (6) concluded that the empirical formula of solanine was still a matter of disagreement, but that it was known to be a

tertiary base with a secondary alcohol group.

Soltys and Wallenfels (26) demonstrated by appropriate methods the presence of a sterol nucleus in solanidine which they suggested was similar to that of cholesterol. The more recent studies of Rochelmeyer, as reviewed by Wolf and Duggar (29), on the homocyclic portion of potato solanidine confirmed this view.

The structure of the nitrogen-containing portion of the molecule, which is still in doubt, was assumed by Soltys and Wallenfels (26) to form a lupinane structure (Fig. 1, A) because of its resistance to degradation, but Clemo, et al, (5) favor another structure (Fig. 1, B) in which a methyl pyrrolizidine is attached to the sterol nucleus at only one point, that is at C^{17}. The structures suggested for solanidine are illustrated in Fig. 1.

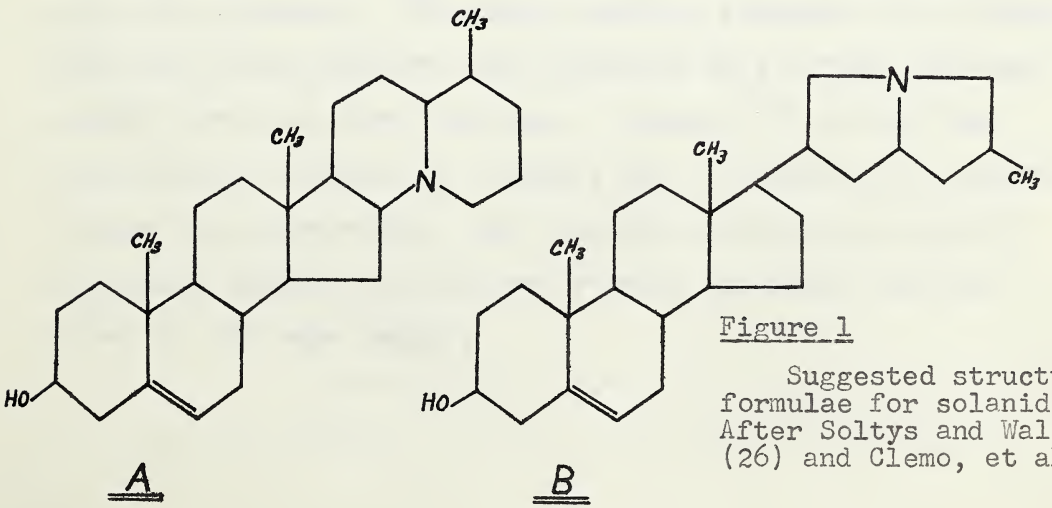
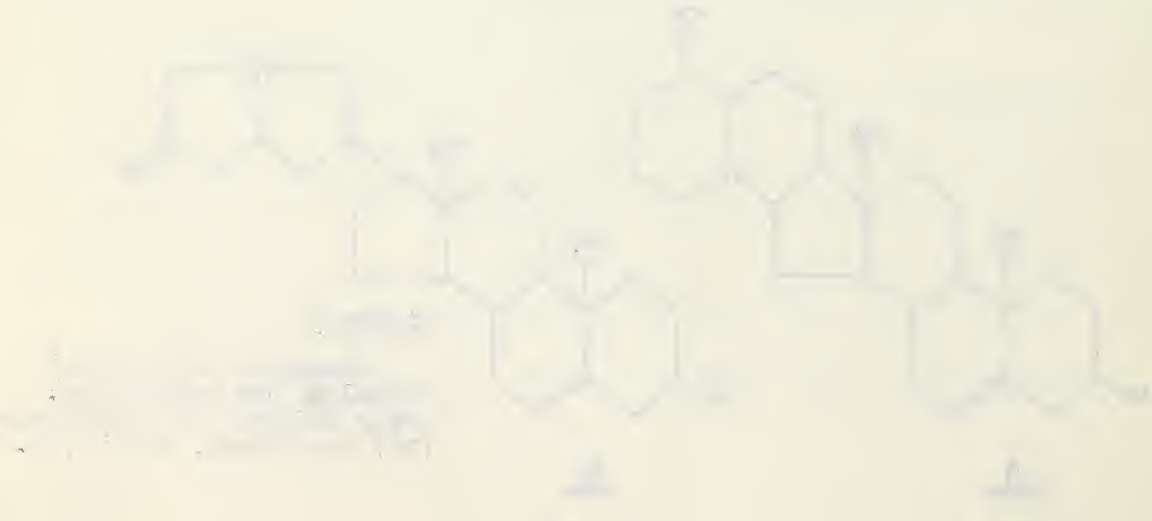


Figure 1

Suggested structural formulae for solanidine. After Soltys and Wallenfels (26) and Clemo, et al (5).



Wolf and Duggar (29) state that in addition to solanine, the two derivatives, solanidine and solanthrene, have been reported in tissues. They say it is not clear that solanthrene occurs as such in tissues; in fact, this substance may be produced as the result of a side reaction by the dehydration of solanidine during the acid hydrolysis of solanine.

Morgenstern (20) determined that 18 table potato varieties averaged 0.0125 per cent solanine, while seven stock varieties averaged only 0.0058 per cent. Yellow potatoes contained less than red or blue varieties. Other workers (24, 29) reported varietal differences, and Lampitt found that solanidine, a basic component of solanine, occurred in free form in sprouts of some varieties but not in others. Gortner (15) states that potatoes normally have about 24 parts per million (0.0024 per cent) of solanine, while Bömer and Mattis (3) give a normal range of 0.002 per cent to 0.0089 per cent solanine. The latter workers suggested that tubers with more than 0.020 per cent solanine were beyond the upper safety limit for food purposes. Lepper (17) showed that even within a variety or a single lot of potatoes the solanine content can vary widely, and variation within the same lot of tubers sampled at different storage intervals has been shown by the same author.

Wolf and Duggar (29), and other workers (3, 19, 20), have noted an association of high solanine and greened tubers, and they conclude that solanine is found in greatest concentration in those organs and tissues where metabolic activity is greatest, such as tops, sprouts, near eyes on the tuber, and in the outer cortex of tubers generally.

The properties of solanine have been determined by several workers (6, 7, 24). It is insoluble in pure water, or practically so. Wolf and Duggar (29) showed that above a pH of 9.3 solanine is quantitatively insoluble in water. It is very slightly soluble in cold ethyl alcohol; quite readily soluble in hot ethyl alcohol; insoluble in ether, chloroform, benzene and petroleum ether. Amyl and ethyl alcohols are used to extract solanine from its alkaline plant juice solutions. It is very weakly basic and does not reduce Fehling's solution, but will reduce certain other copper compounds and sodium thiosulphate. Soltys and Wallenfels (26) reported a melting point of 285° C. Wolf and Duggar (29) reported that an aqueous 0.0379 molar solution of solanine, hydrolysed in four per cent hydrochloric acid, and containing a very slight excess of this acid, "produced a blue fluorescence excited by a considerable range of the ultra-violet region" of the spectrum.

According to Davis and Stadtler (7), "solanine is bitter and pungent, leaving in the pharynx a persistent

acrid sensation." It is poisonous; one-quarter grain being strongly nauseating to a man. It is odorless when dry, but when moist it "exhales" an odor recalling that of cooking potatoes. Henry (16) describes the solanines as "protoplasmic poisons and potent hemolytics," and points out that symptoms of solanine poisoning from potatoes are headache, nausea, emesis and gastritis.

Analytical Methods

(a) Early Work

The most important early work on solanine extraction from potato tubers was that of Morgenstern (20), published in 1907. He used a gravimetric method for the quantitative separation and estimation of solanine.

In 1924, Bömer and Mattis (3) published results of their work on the 1922 and 1923 potato crop in Germany. There had been numerous instances of poisoning from the 1922 potato crop in that country. They also used a gravimetric method of estimation of solanine content. These workers suggested that for very accurate results one should add to the weight of the solanine residue 2.75 mg. for every 100 ml. of liquid used in the precipitations, in order to compensate for the solubility of solanine in alkaline solution.

(b) Later Work

The most important later workers on solanine analysis have been Conner (6), Pfankuch (21), Rooke, et al, (24), and Wolf and Duggar (29).

In 1937, Conner (6) suggested a volumetric method of analysis (based on the determination of the reducing sugars formed on hydrolysis of the complex molecule). This method determines solanine only; in other words, it does not determine any free solanidine which may be present.

In the same year Pfankuch (21) developed a colorimetric method depending on the color produced by the reaction of solanine (and solanidine) with the sulphuric acid-formaldehyde reagent. Rooke (24) stated that Alberti, in 1932, was the first to notice that when solanine was reacted with conc. H_2SO_4 and formaldehyde that a violet-red color was produced. However, Alberti failed to notice that the intensity of the color produced was directly proportional to the concentration of solanine present.

Rooke, and co-workers (24), carried out some rather extensive studies of solanine and its problems for the British Ministry of Food in 1942 and 1943. They tested all the known methods of extraction and determination of solanine. They criticized the early work and said that such workers as Bömer and Mattis, and Morgenstern, applied

methods of extraction of solanine from potato tissue which were subject to error and used methods of determination which were unsatisfactory. All these workers had weighed the small amount of solanine obtained without particular knowledge of the purity of the substance. They also said that Bömer and Mattis had applied too large a correction to their results. Rooke and co-workers obtained a solubility figure of only 0.2 mg. per 100 ml., as contrasted to 2.75 mg. per 100 ml. as given by the earlier workers.

Rooke, et al, (24) said that the method of extraction of Bömer and Mattis, and also the modification of Conner, which necessitated the use of a large quantity of raw material, were found to give a sticky, semi-colloidal precipitate of solanine, difficult to filter and purify. They said that as a method of separation this technique was therefore considered to be both unsuitable and unreliable. Pfankuch's method of extraction was found by these workers to be satisfactory, and his method was used in their work, with some modifications. Rooke, and co-workers, considered Pfankuch's colorimetric method of determination and Conner's volumetric method to be very suitable.

They modified Pfankuch's extraction technique somewhat. Instead of stirring the potato pulp (from 100 - 150 gms. of tubers) in acidified alcohol for fifteen minutes

they allowed the material to steep for 18 hours, with occasional stirring, and then instead of pressing out the juice in a hydraulic press they sucked it to dryness in a Buchner funnel. They used 150 ml. of 95% ethyl alcohol plus 3 ml. of glacial acetic acid for the first extraction and 150 ml. of 64% ethyl alcohol for the second and third extractions. The combined extracts were concentrated in vacuo to about 50 ml., 5 gm. of sodium sulphate added, and the mixture warmed in a water-bath for about one-half hour in order to flocculate the proteins. The mixture was then cooled, and, after the addition of 2 ml. of 20% sulphuric acid, was filtered on a Buchner funnel, the residue being washed with about 10 ml. of water. The filtrate (plus washings) was made alkaline with concentrated ammonium hydroxide, kept overnight at 4° C, and filtered through quantitative filter paper. The precipitate was washed with about 10 ml. of 2% ammonia, the volume of filtrate, plus washings, being noted. The precipitate was then dissolved in 15 ml. of 1% sulphuric acid and the solution diluted with water to 25 ml. Aliquots of this solution were used for the colorimetric, as well as for the hydrolysis method of solanine determination.

For the colorimetric determination of solanine plus solanidine (calculated as solanine), 2.5 ml. of the solanine solution was measured into a small, dry flask. This was cooled in ice, and then, with vigorous shaking, 5 ml.

of concentrated sulphuric acid were added dropwise from a burette. After 1 minute 2.5 ml. of 1% formaldehyde were similarly added, drop by drop. The solution was kept for 90 minutes at room temperature, during which time a purplish-red color developed. The extinction coefficient of this solution was then measured in a Zeiss photometer. The concentration of solanine plus solanidine, expressed as solanine, was obtained by reference to a curve, previously constructed, relating concentration of solanine and optical density. The solanine plus solanidine content of the original was then calculated as solanine, due allowance being made for the solubility (0.2 mg. per 100 ml.) in the ammoniacal solution.

These workers proved that the unpurified solanine isolated in the normal course of determination in potatoes gave a color with maximum absorption at the same wave-length as did pure solanine and pure solanidine indicating that no interfering substance was present. It was found that matching could most easily be carried out at 5300A^{o} and therefore all subsequent extinction coefficients were determined at that wave-length, when a linear relationship was found to exist with the concentration of pure solanine. Rooke and co-workers were able to recover 95% of any pure solanine which they added to potato samples of known solanine concentration.

Wolf and Duggar (29) were able to detect solanine more accurately than this by means of their method

of analysis, using the same colorimetric method. They were able to detect readily as small a quantity as 0.5 mg. of the glycoside in 100 ml. of solution. These workers used a modified Bömer and Mattis procedure for extracting the solanine from tubers. Duplicate samples of approximately 250 gm. of finely minced fresh tuber tissue were weighed out. Weighings were made to within 1 gm. About 250 ml. of 0.25 per cent acetic acid was added, and the mixture allowed to stand at room temperature with occasional stirring for about one hour, after which it was squeezed out through three thicknesses of cheesecloth in a hydraulic press at 5,000 pounds per square inch. The pad of ground tissue was extracted three more times in the same manner. The combined extracts were made slightly ammoniacal as soon as they were prepared, to prevent enzyme activity. Finally, 10 gm. of diatomaceous earth were added and the mixture was well stirred.

The extract was then placed in an oven at about 85° C to evaporate. When only a small amount of the liquid remained, the sides of the container were washed down with a small quantity of 0.25 per cent acetic acid, a slight excess of ammonia was added, the mixture well stirred, and the remaining liquid evaporated off. The dry residue was scraped out of the container, ground in a mortar with a pestle, and extracted with 95 per cent ethyl alcohol in a Soxhlet extractor for approximately 18 hours. The residue was then

reground and extracted with 95 per cent alcohol for another period of 18 hours.

The combined alcoholic extracts were evaporated to dryness and the residue taken up quantitatively with several 25 ml. portions of 0.25 per cent acetic acid. These were filtered, and the combined filtrate was adjusted to a pH of 10.0 to 10.4 with concentrated ammonium hydroxide. The solanine was precipitated from this alkaline solution by digesting over a boiling water bath for 20 to 30 minutes. The alkaloid was collected on a filter paper and purified by redissolving it with approximately 100 ml. of 0.25 per cent acetic acid, adjusting to pH 10.0 to 10.4, as before, with ammonia, and precipitating on the water bath. After three, or at the most, four, such purifications a perfectly white precipitate of solanine was obtained from a clear solution. This was collected on a tared filter paper, washed with three 15 ml. portions of an approximately 2 per cent solution of ammonia, and weighed after being dried to constant weight at 85° C.

As indicated earlier, Wolf and Duggar (29) demonstrated that above a pH of 9.3 solanine was quantitatively insoluble in water. This was probably their most important contribution to the technique of solanine analysis. Thus, by carrying out the precipitation of solanine at a controlled

pH of 10.0 to 10.4, it becomes unnecessary to apply a solubility correction. These men noted that the earlier workers were very inaccurate in their work when they added a solubility correction to their results. They pointed out that this correction factor sometimes exceeded the total solanine actually recovered.

(c) Qualitative Tests Specific for Solanine and Solanidine

Several qualitative tests for the identification of small amounts of solanine and solanidine were used. The three tests (18) most frequently used were: the Alberti reaction, the Brant reaction, and the Bach reaction. These tests are carried out as follows:

(1) Alberti reaction for solanine and solanidine. Dissolve the alkaloidal residue in 1 drop glacial acetic acid, add 1-2 drops concentrated H_2SO_4 and then 1 drop 1% formaldehyde or 0.5% H_2O_2. A purple-red color is produced, which becomes more intense on standing. Saponins and most alkaloids do not give the reaction; morphine reacts with formaldehyde, but not with H_2O_2. Sensitiveness, 0.0025 mg.

(2) Brant reaction for solanine and solanidine. The reagent is a mixture of 0.3 gm. sodium selenate in 8 c.c. water and 6 c.c. H_2SO_4. Warm a little solanine or solanidine with the reagent until a reddish tint appears, then remove from the flame. The mixture gradually becomes raspberry-red. Sensitiveness, 0.025 mg. solanine or 0.01 mg. solanidine.

(3) Bach reaction for solanine. A red color is formed when solanine is added to a mixture of equal parts of concentrated sulphuric acid and alcohol. The color persists for 5-6 hours.

EXPERIMENTAL WORK

- Procedures and Results

For greater ease of presentation, the experimental work has been divided into its various phases.

(a) Pure Solanine Production -

A supply of pure solanine was essential in order that its properties could be studied and so that a calibration factor could be calculated for the particular colorimetric equipment being used. This could not be obtained from any North American or European source; hence it was necessary for the author to isolate and purify his own sample. This proved to be a very time-consuming task. It was found necessary to process a very large volume of potato sprouts in order to obtain a worthwhile yield of the pure alkaloid. During the course of building up the supply of the pure alkaloid, about eighty-five pounds of potato sprouts were processed. About six grams of the pure material were obtained.

The potato sprouts were finely ground in a large food chopper. The resulting mash was placed in two-gallon crocks and enough 2% acetic acid added to cover the material. This was allowed to steep 2-4 days, with occasional stirring. The mash was then pressed under high pressure and the resulting extract was filtered through a

Buchner funnel, using Celite to hasten the filtering process. This filtrate was then made strongly basic with concentrated NH_4OH. The precipitate was digested on a water bath at about 70°C for 20-30 minutes, and then centrifuged for 15-20 minutes. The supernatant liquid was then easily poured off and discarded. The sludgy precipitate was dissolved in 2% acetic acid and heated to 70° C. Some of the proteins were precipitated and were then filtered off. The filtrate was again made strongly basic with ammonium hydroxide and once more digested on the water bath. A large quantity of yellowish-grey precipitate was obtained on Whatman's #42 filter paper. This was dried and then refluxed with 95% ethyl alcohol. The alcohol was filtered while very hot, and upon cooling, the solanine crystallized out in small, loose needles. This was filtered, refluxed and crystallized from alcohol twice more.

The resulting crystals were then refluxed and filtered twice with diethyl ether to remove any traces of solanidine and solanthrene. The resulting solanine crystals were refluxed and crystallized twice more from 95% ethyl alcohol. By now the material crystallized out in snow-white, very tiny needles and had a melting point of slightly over 283° C. The highest recorded melting point for pure solanine is 285° C (26).

(b) Evolution of Standard Analytical Procedure -

The most recent of the solanine analysis methods were studied in the laboratory. The standard analytical procedure eventually used was an amalgamation of the method of Rooke, et al (24), and Wolf and Duggar (29).

The extraction procedure of Rooke was used, and the solanine was precipitated from the acidic extract by adjusting to pH of 10.0 - 10.4 with concentrated ammonium hydroxide, as suggested by Wolf and Duggar. It was found that quantitative procedure was more easily followed when the extract was removed from the potato pulp by means of sucking it through a Buchner funnel than when a hydraulic press was used. Some of the extract was almost invariably lost when using a bulky press. The relatively large volume of solanine precipitate normally recovered in this investigation made it essential that this precipitate be dissolved from the filter paper with 75-80 ml. of 0.5% acetic acid. This extract was then diluted to 100 ml. This was in contrast to the procedure of Rooke, et al (24) who diluted to a final volume of only 25 ml.

The procedure that Wolf and Duggar (29) used with their solanine extracts from tissues other than tubers was followed when producing the violet-red color with concentrated H_2SO_4 and one per cent formaldehyde. The same

proportions of solanine extract, concentrated H_2SO_4 and one per cent formaldehyde were used as was suggested by Pfankuch (21) and Rooke, et al (24), but the volumes differed. The volumes, as used by Wolf and Duggar (29), and in the present investigation, were: 3 ml. of the solanine extract (a precision pipette was used); 6 ml. of concentrated H_2SO_4; and 3 ml. of 1% formaldehyde. Micro-burettes were used to measure accurately the H_2SO_4 and the formaldehyde.

The technique of color formation was that mentioned earlier in this report. A fairly detailed study was carried out with the aim of determining how much time was required for the violet-red color to reach its maximum intensity. This maximum intensity was almost invariably reached between forty and fifty minutes after the last drop of formaldehyde had been added. A typical graph is shown in Figure 2. It is of interest to note that Rooke and his co-workers (24) have stated that ninety minutes is required for maximum color density to be reached, which contrasts sharply with the findings in the present work. After several tests, all of which conformed to the curve shown in Figure 2, the procedure followed was to estimate the optical density of the colored solution with a Fisher Electrophotometer after forty-five minutes, using filter 525. Excellent results were obtained by taking the highest reading obtained during the 45-55 minute time-interval.

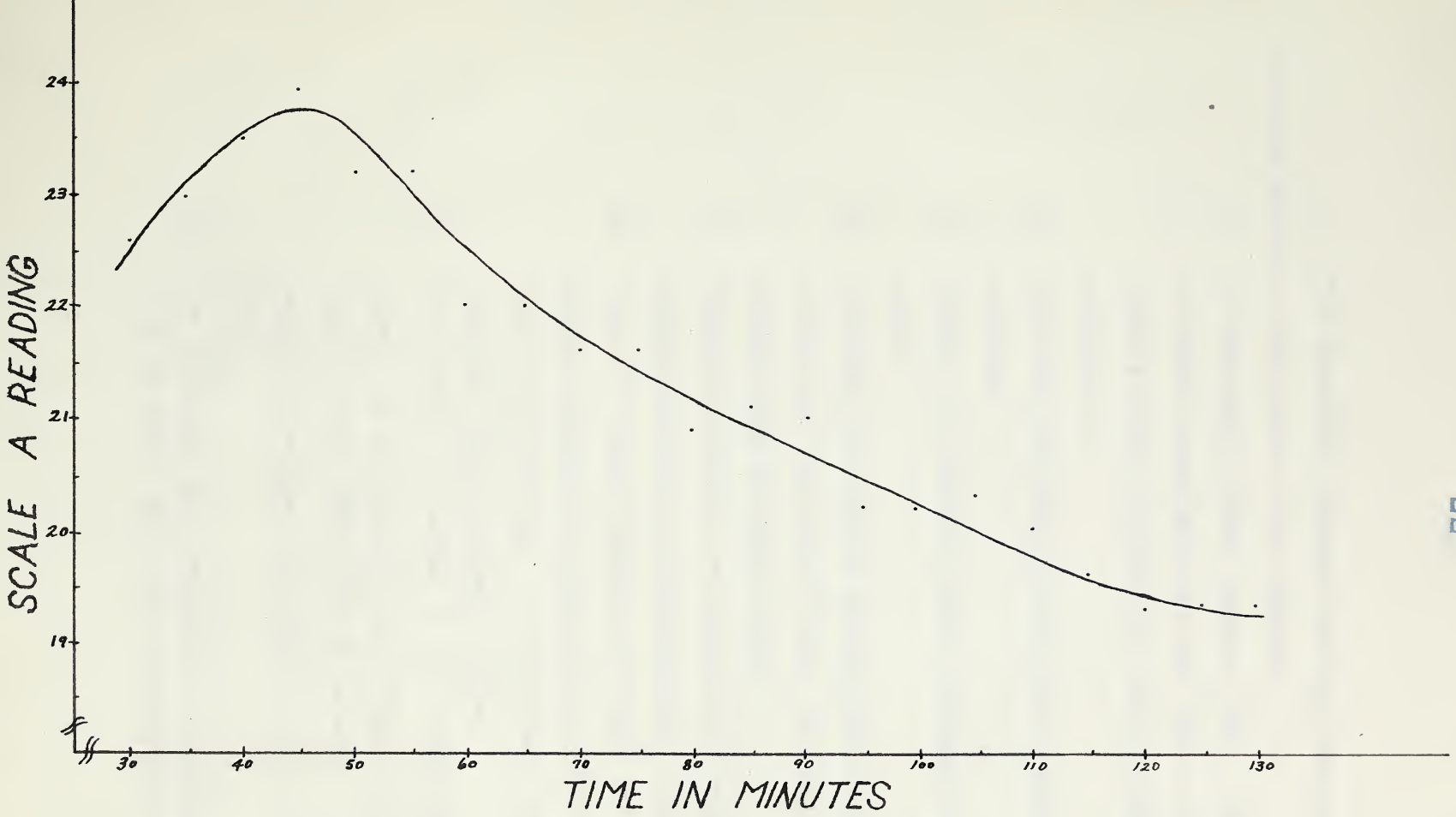


Figure 2

Typical curve showing the relationship of the Scale A reading, of the violet-red color formed by solanine with sulphuric acid and formaldehyde solution, and the time in minutes after the addition of the last drop of formaldehyde.

The method of solanine analysis used as the Standard Method in this study is as follows:

1. To thoroughly minced samples (100 - 150 gms.) of fresh potato material add 150 ml. of 95% ethyl alcohol, followed by 3 ml. of glacial acetic acid.
2. Stir well and keep 18 hours with occasional stirring.
3. Filter on a Buchner funnel (Whatman's #4 paper).
4. A further two similar extractions of the residue are made with 150 ml. of 64% ethyl alcohol (but no acetic acid).
5. Combined extracts are concentrated under reduced pressure to approximately 50 ml.
6. 5 gm. of sodium sulphate are added and the mixture warmed in a water bath at 70° C for about one-half hour. A flocculent precipitate (mostly protein) forms.
7. The mixture is cooled, 2 ml. of 20% H_2SO_4 added, and is filtered on a Buchner with light suction (Whatman's #4 paper). The residue is washed with about 10 ml. of water.
8. The filtrate, plus washings, is adjusted to a pH of 10.0 - 10.4 with concentrated NH_4OH.

9. The solanine is precipitated by digesting in a water bath for 20 - 30 minutes at 70° C; cool.
10. Filter through Whatman's #42 paper and wash the precipitate with about 10 ml. of 2% ammonia.
11. The precipitate is dissolved in three or four 20 ml. increments of 0.5% acetic acid, and made carefully to volume in a 100 ml. volumetric flask.
12. Prepare colored solution according to directions outlined under "Analytical Methods," except that 3 ml. of extract, 6 ml. of concentrated H_2SO_4 and 3 ml. of 1% formaldehyde are used.
13. Determine solanine content after 45 minutes with the Fisher Electrophotometer.

This standard analytical method gave reasonably good results, but its disadvantages were nearly as numerous as any of the methods in common use. Probably the greatest disadvantage was the long period of time required for completion of an analysis. With so much time required, it becomes almost impossible to analyze more than a relatively few samples of tubers. It is also very desirable to reduce greatly the number of steps involved, if possible. When

material must be transferred from one container to another so often, the chances of error are greatly increased.

The conversion of Electrophotometer readings to mg. solanine/100 gm. fresh tuber tissue is developed as follows: For a Scale A reading of 30.0 the extract recovered from 150 gm. fresh tuber material, and using the calibration factor of 0.4266 (determined for the specific instrument used by using pure solanine), the formula is 30 x 0.4266 x 100/150 = 8.53 mg. solanine/100 gm. fresh tuber. Scale A on the Fisher Electrophotometer is a most useful time-saver. It is a logarithmic scale incorporated into the machine. The logarithmic calculations are already taken care of, thus relieving the operator of this burden.

Table I illustrates typical solanine readings in duplicate. As a matter of interest, brief biographical notes are given on the tuber material used. The relationship between tubers noted as "bitter" from cooking tests, and a high solanine content, may be observed.

Table I. Results of Solanine Analyses Using Modified Standard Method

Sample	Duplicate 1	Duplicate 2
A	11.44	10.96
B	36.30	38.06
C	11.04	10.73
D	9.51	9.51
E	37.50	35.80
F	78.32	80.37
G	32.91	31.43

A - 1950 U. of A. Warba tubers, not bitter, extracted September, 1950.

B - 1950 Netted Gem tubers, Southern Alberta source, bitter, extracted early August, 1951.

C - 1950 U. of A. Netted Gem tubers, not bitter, extracted late September, 1950.

D - 1950 U. of A. Netted Gem tubers, not bitter, extracted late February, 1951.

E - 1950 Netted Gem tubers, Southern Alberta source, very bitter, extracted October, 1950.

F - 1950 Netted Gem tubers, Southern Alberta source, very bitter, extracted July, 1951.

G - Early Ohio variety, 1951 tubers, badly sunburned and bitter, extracted mid-September, 1951.

(c) Simple Qualitative Tests on Fresh Tubers -

It was thought that possibly one of the qualitative reagents might give at least a semi-quantitative, rapid test for solanine on fresh tuber slices. Such a rapid test would be most desirable as it could then be used as a quick means of roughly estimating the solanine content of a sample of tubers, either in the field, or immediately after harvest. A detailed analysis could be carried out later on tubers indicating a high alkaloid content.

The Bach reagent was found to produce the best color reaction on thin slices of fresh tubers. It was found necessary to modify the Bach reagent somewhat. One volume of concentrated sulphuric acid to one volume of 95 per cent ethyl alcohol was found to be too harsh. The tuber slices were being charred before any appreciable color had formed. The modified reagent eventually used was: two volumes of concentrated sulphuric acid; one volume of distilled water; and three volumes of 95 per cent ethyl alcohol. It was necessary to cool this mixture before the tuber slices were immersed in it. Also, this reagent must be used fresh; i.e., reagent prepared one day would not produce a color reaction if not used until the following day.

This reagent was placed in small porcelain evaporating dishes and one thin slice of tuber immersed in

each dish. After 25 - 30 minutes a rose-violet color was produced in the slices.

Tests were carried out on slices from tubers of low, medium and high solanine content. The different color intensities produced in preliminary tests gave some reason for optimism, but when more extensive work had been completed on tubers of known solanine content, it was impossible to detect any definite trend. It was therefore decided that further work along this line would be a waste of time.

(d) Simplification of Regular Extraction Procedure -

An attempt was made to shorten the regular extraction procedure by reducing the steeping periods to something less than eighteen hours. Instead of allowing the potato material to merely steep in the alcohol, a variable-speed electrical stirrer was used. While some reduction in the extraction time was possible, it was felt that little, if any, advantage had been gained. It certainly was not the answer to the problem, because at least two days were still required for complete removal of the solanine, at which time there was still a large volume of extract to process. It seemed necessary to extract from a small volume of material, or to devise a simple and short method of estimating the solanine in the extract without the necessity of reduction in volume or of purification.

(e) Fluorescence Studies -

The author was aware that Slatensek and Washburn (25) had devised a rapid fluorometric method for the determination of coumarin in Sweet Clover. It was with considerable interest, therefore, that one particular statement of Wolf and Duggar (29) was noted. They stated that "Solanidine and solanthrene were also prepared by hydrolysing solanine in four per cent hydrochloric acid, and separating and purifying the hydrochlorides of the two bases by appropriate means.

"An aqueous, 0.0379 molar solution of solanine, prepared as indicated above, and containing a very slight excess of hydrochloric acid, produced a blue fluorescence excited by a considerable range of the ultra-violet region. The maximum intensity was at about 300 to 350 millimicrons, and it disappeared at about 227 millimicrons. An alcoholic solution of solanidine, 0.0121 molar, behaved similarly, displaying the same maxima as an aqueous solution of solanine, so that the aglycone alone was responsible for the fluorescence."

It was hoped that perhaps a simple means of estimating the solanine content of a potato extract could be devised, somewhat similar to that used in estimating the coumarin content of a sample of sweet clover. A Coleman

Fluorophotometer was available in this laboratory, and a General Electric ultra-violet bulb was obtained, equipped with a violet filter passing light of about 365 millimicrons, or 3650A°.

A solution of solanine of about 0.04 molar concentration, prepared as indicated by Wolf and Duggar, did give quite a brilliant blue fluorescence when viewed under the ultra-violet light.

An experiment was set up which was designed to determine whether the fluorescence of solanine under ultra-violet light followed a linear pattern. Ten solutions of pure solanine, representing concentrations from 320 mg. to 0.675 mg. per 100 gms. of fresh tissue were hydrolysed in 4 per cent hydrochloric acid. These solutions were cooled, and the intensity of fluorescence determined with the Coleman Fluoro-photometer, using filters B-1 and PC-1. Each of the ten solutions gave a reading of 11 (with the sensitivity control set at maximum), which was the same reading given by a blank of 4 per cent hydrochloric acid.

It had been proposed to use alcohol-extracted potato material (solanine-free) in preparing the blank solution for calibration of the fluorophotometer. Seven samples of this finely pulverized solanine-free material, together with pure solanine, were prepared representing concentrations

from 10 mg. to 1.875 mg. per 100 gm. of fresh tuber tissue. An amount of 0.5000 gms. of each concentration was placed in a dry, 3/4 x 6 inch test-tube, marked for 15 ml. Four per cent hydrochloric acid was added to the 15 ml. mark. These were loosely stoppered and heated in an electric oven at 95° C for two hours. After cooling, the volume was restored to the 15 ml. mark if necessary, using 4 per cent hydrochloric acid. The tubes were well shaken, and the potato material allowed to settle. The degree of fluorescence, of the full-strength extract, was determined using the fluorophotometer. All seven readings were essentially the same, and were very low.

An attempt to determine approximate solanine content by visual means was made. For this trial, a home-made light-proof container for the General Electric ultra-violet bulb was set up. Thin slices of fresh tuber were heated for 30 minutes at 90° C, in four per cent hydrochloric acid, to hydrolyse the solanine present. These slices were then subjected to the 365 millimicron ultra-violet wave-length. Using tuber slices from potatoes of widely differing solanine content, it was not possible to estimate differences in intensity of fluorescence.

As a point of interest, all tuber slices showed slight fluorescence, and it was observed that the area of maximum fluorescence was immediately beneath the tuber skin.

These disappointing and negative results forced the conclusion that the degree of fluorescence of solanine in ultra-violet light was not intense enough to be used as a means of solanine estimation from a potato extract.

(f) Extraction from Dried Material -

Many of the short, alkaloid estimation techniques use the dried leaf or root material (10, 23). An attempt was made to devise a shortened method of solanine estimation patterned somewhat on these methods.

The fresh potato tubers were sliced longitudinally down the centre. One-half was analyzed for solanine content, and the other half was sliced thinly and dried on a wire screen to constant weight at 65° C. The dried slices were finely pulverized with a mortar and pestle. This dried and pulverized material was used in an effort to extract solanine, using a Soxhlet type extractor. Two solvents were used: 95 per cent ethyl alcohol, and 2 per cent hydrochloric acid.

Ten grams of the dried tuber material were placed into a 30 x 80 mm. Soxhlet thimble, the apparatus assembled, and 125 ml. of 95 per cent ethyl alcohol used as the solvent. Various extraction periods, from five to fifteen hours, were tried. In no instance could any definite test for solanine be obtained from the extract.

Two per cent hydrochloric acid was similarly used as a solvent. Solanine is very soluble in dilute acids, and also solanine is easily hydrolysed in hydrochloric acid to solanidine and its three sugars. It was hoped that by refluxing the dried and pulverized tuber material in the Soxhlet extractor that the solanine could be extracted by means of the fresh, hot solvent; and at the same time, the simmering extract in the distillation flask would become hydrolysed. Theoretically, the aglycone could then have been precipitated with ammonium hydroxide and taken up in an alkaloid solvent such as chloroform or diethyl ether.

Only a trace of solanidine could be detected from any of the extracts; far below the amount known to have been present in ten grams of the dried tuber material. It was presumed that the solanine present must have been altered somewhat during the drying process, and also some of the solanidine was probably destroyed during the HCl extraction.

At this point in the investigation, it appeared that it would be necessary to extract the solanine from fresh potato tissue. Actually, this was desirable, because the drying of the tubers necessitated another step, in the already involved technique. This line of thought led to the next phase of the investigation.

(g) Soxhlet Extraction of Fresh Material -

The first extraction by this method was tried on a sample of 1950, University of Alberta grown, Netted Gems. They were finely macerated in a Waring Blendor, and a 25.0 gm. sample was carefully transferred to a 30 x 80 mm. Soxhlet extraction thimble. About 150 ml. of 95 per cent ethyl alcohol were added to the distillation flask, and five drops of glacial acetic acid were added. The apparatus was assembled and placed on an electric hot-plate. Extraction was continued for eight hours.

The resulting extract was reduced in volume, partially purified and the solanine precipitated in a manner similar to the technique used in the Standard Method of extraction and estimation. The acetic acid solution of solanine was diluted to volume in a 25 ml. volumetric flask. The solanine content of this extract was estimated by measuring the optical density of the violet-red color, produced by the sulphuric acid-formaldehyde reagent, in the Fisher Electrophotometer.

A solanine concentration of 4.48 mg. per 100 gms. of fresh tissue was indicated from this extract. The solanine content of a sample of these tubers taken from the same container (but not the same "mix"), and determined by means of the Standard Extraction Method, was 9.51 mg. per 100 gms.

A second Soxhlet extraction, similar to the first, and differing only in the extraction time, was carried out. This sample was extracted for fifteen hours. A solanine concentration of 11.30 mg. per 100 gms. was indicated. This was encouraging, as a higher solanine content was indicated than that which was obtained using the longer method.

Several more single determinations were made, all with gratifying results. The technique was altered somewhat and improved. A series of duplicate determinations were then made, using two identical sets of Soxhlet apparatus. Very good checks were obtained with the duplicate extractions.

A series of extractions were planned in an attempt to determine the minimum extraction period needed to remove completely the solanine from the potato sample. Tables II, III and IV contain the results of these extractions, two colorimetric determinations having been made on each duplicate extract. Solanine contents indicated in all tables are mg. of solanine per 100 gms. of fresh tuber tissue. The three sets of duplicate extractions in Tables II, III and IV were carried out on samples weighed from the same "mix" in the Waring Blendor. The solanine concentrations reported in Table II are for a different sample of tubers than those reported in Table III; and likewise for Table IV.

Table II

Extraction Time in Hours	Solanine Content (mg./100 gm.)
15	8.19
17	8.19
19	9.39

The increased amount of solanine obtained with the 19-hour extraction time led to a further similar trial using extraction periods of 18, 20 and 22 hours.

Table III

Extraction Time in Hours	Solanine Content (mg./100 gm.)
18	7.68
20	7.76
22	7.85

Although the amounts of solanine obtained from each of the 18, 20 and 22-hour extraction periods were essentially the same, there was a small, but consistent, increase indicated from each of the two longer extraction periods. Another set of duplicate analyses was then carried out, using extraction periods of 20, 22 and 24 hours.

Table IV

Extraction Time in Hours	Solanine Content (mg./100 gm.)
20	46.58
22	46.58
24	40.02

These figures indicate that 20 hours should be taken as the minimum extraction period, and 22 hours should be considered the maximum period. The loss of solanine in the 24-hour extraction period may possibly be due to a small amount of solanine breakdown because of an excessively long boiling period in the acidified alcohol.

Table V contains comparative solanine contents of several samples of potato tubers as determined concurrently on the same "mix" by means of the Short Soxhlet Method and the Long Standard Method.

Table V

Number of Sample	S O X H L E T *		S T A N D A R D	
	Duplicate I	Duplicate II	Duplicate I	Duplicate II
A	35.07	34.13	32.91	31.43
B	37.20	36.52	34.75	34.35
C	66.18	65.44	68.34	67.83
D	7.34	7.68	3.50	4.05
E	46.88	46.28	44.79	41.98

* Samples B and C were extracted for 17 hours, while A, D and E were extracted for 20 hours.

These data indicate that the Soxhlet Method of extraction gives more consistent checks between duplicates, and usually indicates at least a slightly higher alkaloid content than does the old long method. In at least one instance the Soxhlet Method indicated approximately twice as much solanine as did the long method. No reason could be found for this evident failure of the long method to extract more than about half of the alkaloid which was present. Another worker at the University of Alberta experienced this same inconsistency in results with the Long Standard Method of extraction and obtained very good results using the Short Soxhlet Method.

Table VI contains the results of a series of duplicate Soxhlet extractions. Each figure is an average of two colorimetric determinations carried out on each extract.

Table VI

Duplicate I	Duplicate II
37.20	36.52
16.55	17.75
46.88	46.28
66.18	65.44
46.88	46.28
50.28	49.54

Table VI (cont'd)

Duplicate I	Duplicate II
17.66	18.17
8.11	8.28
8.45	7.93
7.34	7.68
7.93	7.76
7.68	7.85
7.68	7.68
25.68	26.02
35.07	34.13
40.31	39.72

Treating these solanine figures as "Paired Data" a non-significant "t" value of 0.03 is obtained. This indicates that the normal populations sampled have the same means and the same variances; or, in other words, the two samples are randomly drawn from the same normal population. It can therefore safely be said that any of the variations between the duplicate analyses are attributable to normal experimental error and not to errors inherent in the method itself.

The standard error (standard deviation of means) of the method $\frac{(s)}{(\sqrt{n})} = \pm 0.154$ mg. of solanine per 100 gm of fresh tuber tissue.

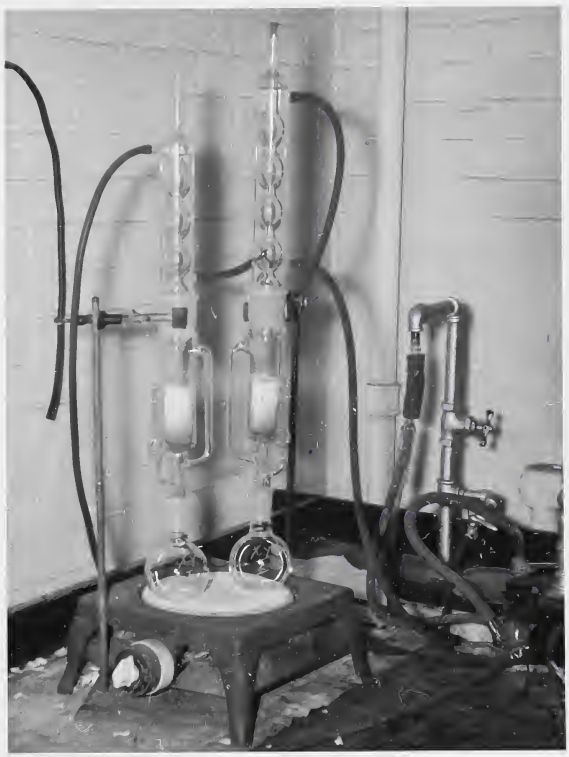


Figure 3

Extraction apparatus used for the Soxhlet Extraction Method of determining the solanine content of fresh potato tubers.

Figure 4

Micro-burettes and ice-bath used in the production of the violet-red color with sulphuric acid and formaldehyde.

Figure 5

Color photo showing the color changes which a solanine solution undergoes. From left to right: concentrated H_2SO_4 added; immediately after addition of the 1 per cent formaldehyde; fifteen minutes after addition of last drop of formaldehyde; forty-five minutes after addition of last drop of formaldehyde.

The Soxhlet extraction technique is as follows:

(1) Tubers are washed, and allowed to dry.

(2) They may be coarsely ground in a food chopper or sliced thinly and then transferred to a Waring Blendor. Here the tuber material is very finely pulverized.

(3) Duplicate, 25.0 gm. samples are carefully weighed in 50 ml. beakers.

(4) The tuber material is transferred to a 30 x 80 mm. Soxhlet thimble (the thimble being held in a second 50 ml. beaker) with a stirring rod equipped with a rubber policeman.

(5) The beaker is washed once with a few ml. of 95 per cent ethyl alcohol, from a wash bottle. This first washing is transferred to the Soxhlet thimble.

(6) The thimble is carefully lowered into the Soxhlet apparatus.

(7) Both beakers are thoroughly washed with several small increments of 95 per cent ethyl alcohol (25-30 ml. of alcohol are used in washing the two beakers). The washings are transferred to a 250 ml. distilling flask.

(8) A further 100 ml. of 95 per cent ethyl alcohol is added to the flask, plus five drops of glacial acetic acid.

(9) The Soxhlet apparatus is assembled and placed on a hot-plate to extract. The equipment used must be equipped with perfectly fitting ground-glass joints.

(10) Extraction is continued for 20-22 hours. The hot-plate temperature should be adjusted so that the alcohol is just boiling.

(11) The extract is reduced in volume to 10-15 ml., in vacuo, in the distilling flask. For this purpose a two-holed rubber stopper is fitted with an open glass rod and a fine capillary tube. The capillary tube is fitted with a short length of rubber tubing which can be clamped with a pinch-cock. In this manner, a very slow stream of tiny air bubbles is allowed to enter the distillate at the bottom of the flask. This serves to keep the extract boiling without violent bumping.

(12) 1.0 gm. of Na_2SO_4 is added to the distilling flask; it is swirled vigorously, and heated in a water-bath at 70° C for 20-30 minutes. A flocculent precipitate of protein material forms.

(13) The extract is cooled, 0.5 ml. of 20 per cent H_2SO_4 is added, and the extract filtered through a 9 cm. Whatman's #4 paper. 10-15 ml. of 0.5 per cent acetic acid (from a wash-bottle) are used to wash the distilling flask and the filter paper.

(14) The filtrate is allowed to run into a 100 ml. beaker. The pH is adjusted to 10.0 - 10.4 with concentrated NH_4OH; a Beckman glass electrode, pH meter is used to measure the pH.

(15) The precipitate is flocculated by heat at 70° C in a water bath for 30 minutes and is then cooled. The precipitate can be filtered off at this stage, or it may be kept in a refrigerator over night before being filtered. Equally good results have been obtained using either of these two practices.

(16) The solanine precipitate is filtered off through a 9 cm. Whatman's #42 paper, using a small funnel. Precipitate is washed with about 10 ml. of 2 per cent NH_4OH, preferably applied in two increments of 5 ml. each.

(17) When no smell of ammonia remains on the filter paper, it is transferred to the same 100 ml. beaker used earlier, and the solanine

is dissolved in several small increments of 0.5 per cent acetic acid.

(18) The acetic acid solution of solanine is filtered through a Whatman's #12 folded filter paper into a 50 ml. volumetric flask. The paper is washed with 0.5 per cent acetic acid sprayed from a wash-bottle, and the volume is carefully made to the 50 ml. mark using 0.5 per cent acetic acid.

(19) The solanine concentration is determined by reading the optical density of the violet-red color, produced by the sulphuric acid-formaldehyde reaction, in the same manner as was done with the long Standard Method (see pages 12 and 20).

It has been possible, using the above procedure, to complete a duplicate analysis in just over a day and a half. This is allowing for the 20-hour extraction period, and also allows for an overnight delay at the precipitated-solanine stage. Most of the actual extraction takes place over-night, and at no time does the Soxhlet apparatus require more than just a cursory examination to see that the hot-plate is operating and to be certain that the cold water is flowing through the condensors. The small volume of extract (about 130 ml.) reduces to the required

volume quickly under a vacuum. The resulting very small volume of extract, plus washings, normally filters rapidly, so that very little time is wasted. It is only when tubers with a very high solanine, and protein, content are being analysed that the filtering process may be delayed somewhat. It was felt that 25 ml. of solvent was insufficient to remove completely the solanine from the filter paper, so 50 ml. was used. This added dilution of the extract is taken into account when the Electrophotometer readings are converted to mg. solanine.

This analytical technique has made it possible to reduce the time factor to approximately one-third of that required by the old method. Also, the number of individual steps involved has been greatly reduced. Standard quantitative analytical procedure can easily be followed throughout the entire extraction and purification. It was felt that any precision lost because of reducing the weight of fresh tissue to 25.0 gms. was more than compensated for by the reduction in the number of individual steps and cumbersome transfers of large volumes of material.

Wolf and Duggar (29) showed that the colored solution formed by solanine with concentrated sulphuric acid and one per cent formaldehyde exhibited very close agreement with Beer's Law. This agreement was particularly close over

the range of 5-10 mg. per 100 ml. of solution. This range (5-10 mg. per 100 ml.) corresponds very closely to the most sensitive area of the Fisher Electrophotometer scale. Therefore, solanine solutions of higher concentration than this were diluted with 0.5 per cent acetic acid until their concentrations fell within this range. This colorimetric method of estimating the alkaloidal content of a potato extract determines both solanine and solanidine. This combined solanine and solanidine content was, in each instance, reported as mg. solanine.

A filter passing light of 5300A° was not available for the electrophotometer used, so a 5250A° filter was employed.

DISCUSSION OF RESULTS

The data presented bear evidence that a new analytical technique, for the quantitative determination of solanine in potato tubers, has been devised. A method such as this has been urgently required, at this institution at least. This shorter, easier-to-handle, method should prove to be of considerable value to future workers in this field. It will be possible to analyse up to three times as many tuber samples in the same length of time as was possible with the older methods.

CONCLUSIONS

The negative nature of most of the trials reported herein bears out the complexity of solanine analytical procedure indicated by previous workers. Detailed investigation has shown several unsuccessful avenues of approach, which should constitute a record of value to further work. The much-shortened Soxhlet analytical method represents what is felt to be a very significant improvement in the procedure for quantitative determination of this poisonous and troublesome glycoalkaloid. If, as might be anticipated, irrigation and modern machine cultural methods of potato production encourage the setting of tubers near the ground surface, increases in tuber bitterness may result. A short analytical procedure may, therefore, contribute much to studies of causal factors and control measures.

ACKNOWLEDGEMENTS

This research project was carried out under the direction of Dr. R. J. Hilton in the Research Laboratory of the Department of Plant Science, University of Alberta. The investigation has been financed by means of a Bursary held by the author and awarded by the National Research Council of Canada. Grateful acknowledgement is made to the Council for this assistance. Important equipment also was purchased for the work through a National Research Council grant. Technical assistance from Mr. F. Gleave, Department of Physics, and Professor R. Murray, School of Pharmacy, both of the University of Alberta, also is acknowledged with thanks.

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